

Title:

Suboptimal Exposure to Anti-TB Drugs in a TBM/HIV+ Population is not Related to Anti-retroviral Therapy

Authors ME Török¹, G Aljayyousi^{2*}, Waterhouse D², TTH Chau³, NTH Mai³, NH Phu³, TT Hien³, Hope W⁴, JJ Farrar³, Ward SA².

Affiliations

1. University of Cambridge, Department of Medicine, Box 157, Addenbrooke's Hospital, Hills Road, Cambridge, United Kingdom
2. Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, United Kingdom
3. Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Hi Chi Minh City Vietnam
4. University of Liverpool, Sherrington Building, Liverpool, L69 3GE

Correspondence

* G Aljayyousi, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, United Kingdom

Tel: +44(0)151 705 3100

Email: ghaith.aljayyousi@lstmed.ac.uk

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ABSTRACT

A placebo-controlled trial that compares the outcomes of immediate versus deferred initiation of antiretroviral therapy in HIV+ve Tuberculous Meningitis (TBM) patients was conducted in Vietnam in 2011. Here, the pharmacokinetics of Rifampicin, Isoniazid, Pyrazinamide and Ethambutol were investigated in the presence and absence of anti-HIV treatment in 85 patients. Pharmacokinetic analyses show that HIV therapy has no significant impact upon the pharmacokinetics of TB drugs in this cohort. The same population, however, displayed generally low CSF and systemic exposures to rifampicin compared to previously reported HIV –ve cohorts. Elevated CSF concentrations of pyrazinamide on the other hand were strongly and independently correlated with increased mortality and neurological toxicity. The findings suggest that the current standard dosing regimens may put the patient at risk of treatment failure from suboptimal rifampicin exposure, and potentially increasing the risk of adverse CNS events which are independently correlated with pyrazinamide CSF exposure.

INTRODUCTION

Tuberculosis (TB) is the most common opportunistic infection in HIV-infected individuals. Tuberculous meningitis (TBM) is the most devastating form of the disease and is expected to develop in 1% of Tuberculosis cases¹, and in 2015, 10.4 million cases of TB were identified². TBM results in death or disability in more than half of affected patients³. A strong association between HIV and TB has been shown globally where it is estimated that 25% of TB deaths could be related to HIV⁴ and the viral infection has been shown to increase the risk of developing TBM (10% of HIV -ve patients with latent TB are expected to develop TBM in their life time, but if the patient is HIV+ve they would have a 10% chance to develop TBM every year⁵). HIV coinfection with TBM is also associated with higher mortality⁶. The treatment of HIV infection requires long-term antiretroviral therapy (ART), which may result in drug-drug interactions with many other drug classes, including antituberculosis (anti-TB) agents. There are concerns about altered pharmacokinetics of anti-TB drugs that are concomitantly administered with ART which could adversely affect the efficacy or toxicity of some these drugs in this patient group. A discrepancy exists in the literature on the interactions between ART drugs used in HIV and anti TB drugs. The timing of ART initiation in patients presenting with TBM has been a subject of debate where it has been hypothesised that delaying ART initiation can reduce the clinical relevance of drug-drug interactions which could in turn lead to adverse effects such as IRIS (increased immune reconstitution inflammatory syndrome)⁷. Conversely early initiation of ART has been positively correlated with AIDS free survival in patients with HIV-associated TB⁸. A debate also exists on the interaction between anti-retroviral drugs and Rifampicin in particular with the literature providing mixed messages on whether anti-retroviral therapy decreases Rifampicin exposure or doesn't affect it at all^{9, 10, 11}.

Although first line anti-TB drugs have very well characterised pharmacokinetic (PK) profiles in cohorts of people suffering pulmonary TB or in healthy subjects, the PK of these drugs and their CNS exposure levels are poorly understood in HIV +ve patients with TBM. The current study investigates the population pharmacokinetics of first-line anti-TB drugs in a unique cohort of Vietnamese patients presenting with HIV-associated TBM, in order to establish whether there are indeed unacceptable drug-drug interactions with ART and to investigate any significant relationships between drug exposure and clinical outcomes which is defined here as survival beyond 9 months and survival beyond three years.

RESULTS

Pharmacokinetic analyses

Table S1 summarises the number of subjects, the number of Ethambutol (ETB), Isoniazid (INH), Pyrazinamide (PZA) and Rifampicin (RIF) plasma concentrations determined, and the number of concentrations per subject available for analysis. Parameters arising from the PK analysis as well as average CSF drug levels (which are in this case mostly free drug levels in contrast to plasma drug levels which are total drug) reported in Table S1 for all four drugs. Monte-Carlo simulations based on the achieved parameters were generated for 1000 simulated subjects. The simulations show that greater than 95% of patients fall within the 5% and 95% percentiles of the simulation, indicating that the parameters generated were sufficient to explain the data (Figure 1).

ETB exposure levels in this study (Table 1) were consistent with previous values reported in HIV-uninfected, healthy subjects or patients with pulmonary TB (Table 2). In contrast the INH CL/F value was higher than most reported data from HIV-uninfected adults (Table 2). Exposure levels of INH also displayed a high degree of variability (66.5% on CL/F), which was expected given the presence of both fast and slow acetylators within our patient cohort¹².

The pharmacokinetic profile of PZA was consistent with data published in the literature in healthy volunteers and adult TB patients (Table 2). PZA displayed the highest CSF exposure out of all drugs, with the average CSF concentration at two hours post dosing of 15.6µg/mL which is only two- to three-fold lower than the C_{max} in the systemic circulation.

The exposure levels of RIF (assessed as the CL/F value) were markedly lower than reported exposure levels in the literature (Table 2) with a CL/F value of 17.9 L/hr compared to an average of 10.0L/hr in various studies in HIV-uninfected subjects. CSF exposure was particularly low with an average CSF concentration of 73ng/mL, a value substantially lower than that observed in HIV-uninfected TBM patients receiving a similar or even a lower dose of RIF (300mg) in most cases¹³.

Covariate Analysis.

Effect of ART initiation time on the exposure of anti-TB drugs:

We investigated if the timing of ART initiation affected the exposure to any of the four anti-TB drugs.

Estimates of steady state exposure quantified as area under the curve (AUC) were compared among those who had immediate ART and those who had deferred ART initiation (at 2 months). The results show consistent exposure between patients that is not influenced by immediate or concomitant ART (Figure 2, A.). CL/F means were very similar across two groups (RIF: $18.3\text{L/h} \pm 8.1$ and $17.3\text{L/h} \pm 8.0$, PZA: 2.7 ± 0.7 and 2.4 ± 0.57 , INH: 25.3 ± 15.8 and 25.6 ± 16.5 , ETB: 51.8 ± 15.3 and 56.2 ± 14.8 for immediate and delayed ART initiation, respectively, $n=19-26$).

A similar analysis was performed to compare the CNS exposure to anti-TB drugs (as assessed by the CSF concentration at two hours post dose) in those who had immediate or delayed ART. The results were consistent between the two groups for all drugs except for INH which exhibited a two-fold increase in CSF levels when concomitantly administered with ART. CSF concentration at 2 hours after dose administration were as follows: (RIF: $78.6\text{ng/mL} \pm 57.6$ and $65.8\text{ng/mL} \pm 41.4$ ng/mL, PZA: $15.5\text{mg/L} \pm 7.2$ and $15.6\text{mg/L} \pm 7.7$, INH: $448.2\text{ng/mL} \pm 361.6$ and $210.6\text{ng/mL} \pm 221.8$, ETB: 171.7 ± 166.5 and $146.1\text{ng/mL} \pm 113.3$ for immediate and delayed ART initiation, respectively, $n=34-46$) (Figure 2, B.)

Effect of Drug exposure upon study outcomes

We studied the correlation between systemic exposures of each drug as defined by AUC level at steady state and clinical outcomes, including mortality and neurological events and other key parameters as indicated in Table 3. Variations in systemic exposure to any of the four drugs did not seem to affect clinical outcomes.

We also studied the relationship between CNS exposure of anti-TB drugs and clinical outcomes including neurological events and death. Interestingly, both ETB and PZA exposures correlated with mortality rates and neurological events. While the association between mortality rates and ETB concentrations was limited, albeit significant (Table 3), PZA CSF exposure strongly correlated with mortality (figure 3 A and B, Spearman's coefficient with time till death = -0.47 , $P<0.0001$). Both drugs' CSF exposure correlated with the incidence of neurological events suffered by patients (Table 3). In a binomial logistic regression analysis that has been performed with this data, only PZA was shown to have an independent effect when controlling for other factors as described earlier.

DISCUSSION

The optimal timing of ART initiation in patients with HIV-associated TBM has been a subject of considerable debate. In patients with pulmonary TB early ART initiation is associated with improvements in AIDS-free survival, particularly in patients with low baseline CD4 counts²⁷. In patients with HIV-associated TBM, however, our previous study suggested that immediate ART did not result in a survival benefit, but was associated with an increase in severe adverse events²⁸. The current study aimed to investigate the effect of ART initiation time upon drug exposures both in the systemic circulation and the CNS, and to assess if drug exposure correlated with clinical outcomes. We also examined the overall pharmacokinetics of anti-TB drugs in the circulation and CSF of patients with HIV-associated TBM.

A few studies have previously measured the outcomes of concomittant ART and anti-TB drugs in HIV-infected patients with pulmonary tuberculosis. However, the pharmacokinetics of anti-TB drugs were never studied in a TBM infected population that is also HIV +ve and this to our knowledge, is the first study to systematically examine anti-tubercular drug pharmacokinetics in HIV+ve patients with TBM. Studying the pharmacokinetics in such a population is of importance as the pathology with the concomittant infection is severe and it's not unlikely that PK properties could change dramatically in such conditions. Previous studies in TB (and not TBM) patients who are HIV +ve have shown conflicting results. Bhatt et al.⁹ found that antiretroviral drugs did not influence exposure to RIF or INH when concomittantly administered in HIV-infected TB patients. Other studies, however, have found that HIV-infected TB patients receiving ART often have a lower exposure to RIF and INH^{10, 11} compared to HIV-negative individuals.

In the current study of HIV-infected TBM patients we found lower exposure levels of RIF than previously reported in either healthy individuals, or in adults with tuberculosis, whereas PZA, ETB and INH pharmacokinetics were consistent with earlier data from healthy adults and in those with pulmonary tuberculosis. This decreased exposure of RIF however, was not related to drug-drug interactions as the observation was independent of concomittant ART administration. It is worth noting that in this study however, the duration of the intensive phase where ETB and PZA are administered was longer than the usual 8 week period.

We used AUC as a universal metric for drug exposure as drugs were administered at various dosing brackets that are not perfectly proportional to body weight which means that drug exposure is not perfectly correlated with Clearance values. AUC/MIC has been generally an accepted measure of pharmacodynamic targets for the anti-tubercular drugs investigated in this study²⁹, and as the work

here aims at comparing exposures of this population to other standard populations as well as studying the relationship between exposure and various clinical outcomes, AUC was used as the primary PK variable and was correlated to all other variables. Other PK parameters that would relate to time above MIC (such as rate of elimination) were also studied but not reported as they don't add anything new to the information provided by AUC.

The relatively high clearance values observed for RIF are of particular concern and suggests that increased dosing of the drug is required in patients who are HIV-infected, regardless of whether they are receiving ART or not. CSF exposure levels to RIF were also remarkably low, with an average CSF concentration at two hrs not exceeding 72ng/mL, which is far below the RIF MIC for most *M.tuberculosis* strains, which are reported to be in the 200-400ng/mL range³⁰. Importantly, CNS exposure to RIF after 10mg/kg dosing in this population is lower than what would be expected in other populations receiving the same dose³¹. This has significant implications for optimising RIF dosing regimens in this particular population, where the infection is within the CNS and can play a role in driving TB resistance to RIF. It is important to notice however, that only 2h time points for CSF were available for all patients which adds some uncertainty to whether those concentrations are truly representative of low CSF exposure to RIF, although the 2h concentrations in this study were compared to only 2h CSF time points in the literature from which conclusions were drawn.

Low systemic exposure levels to RIF have been recently shown to have a direct correlation with mortality rates in TBM patients with high exposure levels (600mg IV) being associated with significantly lower mortality than low exposure levels (450mg orally)²¹. A recent study has investigated, in children with TBM, the CSF exposure to anti-tubercular drugs and found that at a dose of 10mg/kg, rifampicin levels are lower than the minimum inhibitory concentration of susceptible bacteria³². The reasons behind the low RIF exposure in this cohort are unknown, and whether HIV status is the driving force behind the low exposure has been a subject of debate as previously discussed in the introduction. Other reasons might include wasting or malnutrition and associated issues such as oedema in the small intestines, or bacterial over growth which have been reasons that were suggested in the past^{33, 34}.

We performed Monte-Carlo simulations based on the parameters generated from this cohort to simulate what dose would be needed to achieve the exposure expected from a standard 10mg/kg dose in an average HIV-negative adult population. Literature PK data for RIF in HIV-negative subjects have been averaged from papers that reported both CL/F and V/F values²⁵) and an average inter-individual variability of 30% has been assumed on all parameters. These simulations were then

compared to RIF exposure in the current cohort (Figure 4). The simulations reveal a significant discordance between the exposure in the current cohort and exposure in pulmonary TB patients receiving a typical 600mg RIF treatment daily (Figure 4, A). The data generated suggest that a 900mg oral dose would be more appropriate in this patient cohort based on the assumptions of the model (Figure 4, B). Increasing RIF dose in fact significantly improves survival rates among TBM patients according to the latest clinical studies where it has been shown that a high dose of 600mg RIF administered intravenously to TBM patients significantly improved the clinical outcome for these patients²¹. Additionally, according to literature data³¹, the systemic exposure that would be anticipated to be achieved in the current cohort with a higher 900mg dose is predictive of CSF exposures that exceed the required TB MIC value of RIF in the CSF. However, a recent trial of intensified therapy for TBM, which included higher-dose rifampin (15 mg per kilogram per day) and levofloxacin (20 mg per kilogram per day) for the first eight weeks of treatment, failed to demonstrate and improvement in survival³⁵. This indicates that RIF exposure might not be the only variable that is driving higher mortality rates in such population, but the low CSF exposure of RIF in the CSF which is below its MIC levels must be still considered in any optimised dosing regimens for the disease in similar populations.

INH also exhibited lower exposure levels in this cohort when compared to HIV-negative subjects. However, for INH the average CSF concentration at 2 hours (347.1ng/mL) is some 10-20 fold higher than its MIC value of 20-40ng/mL³⁰.

In the current study we found a strong correlation between high CSF PZA concentrations and high mortality rates ($P < 0.004$, Figure 3, A.). There was also a correlation between the average CSF PZA concentration and the incidence of neurological events (Figure 4, B). CSF PZA concentrations did not correlate with measures of BBB integrity such as CSF protein concentrations ($p = 0.34$, $n = 83$).

ETB CSF exposure showed a similar trend but with a weaker correlation with mortality (Spearman's coefficient = -0.266 , $P = 0.017$) although higher CSF concentrations of ETB were more strongly predictive of an increase in neurological events (Spearman's coefficient = -0.358 , $P < 0.001$) (Table 3). We used binomial logistic regression to study the correlation between both PZA or ETB as well as other markers of the CSF environment with events of mortality as described in the methods section. Only PZA showed a significant effect upon mortality in three years (Sig. = 0.013), all other CSF markers including RIF, ETB or INH CSF levels did not reach to significance levels in the logistic regression model and were not correlated to PZA exposure and were hence not discussed further.

PZA has not been associated with CNS toxicity previously³⁶. Decreasing PZA doses by half in this cohort would still achieve CSF concentrations that are well above the PZA MIC value of (512-1024ng/mL)³⁶ against *M. tuberculosis* but is expected to bring PZA down to levels that are not correlated with high incidence of mortality. Additionally, CSF ETB concentrations (average = 160.8ng/mL) were far below the MIC levels (500-1000ng/mL)³⁶ which poses the question if its inclusion in TBM therapy is worthwhile, particularly given its marginal association with CNS toxicity.

One of the main aims of this study was to look at the effect of concomitant ART upon the PK of anti-TB drugs. We did not find any significant drug-drug interaction between the anti-TB drugs caused by these ART drugs. The only variable that changed on ART initiation time was the CSF exposure to INH which was reduced by half when ART is given concomitantly (Figure 2). However, CSF INH concentrations were well above MIC levels in both study arms.

In conclusion, there are three main findings that the work outlined herein has identified: Firstly, Antiretroviral therapy does not significantly interact with or change the exposure of any of the TB-drugs which indicates that the timing of anti-retroviral therapy is irrelevant from a pharmacokinetic point of view. Secondly, RIF displayed particularly low exposure in this cohort in comparison to other populations which would question the current dosing regimens of RIF in TBM/HIV+ve populations and underlines a need to evaluate increasing RIF doses to achieve optimal exposures. Low RIF levels were a particular concern as they have been associated with increased mortality in previous reports²⁶ Thirdly, Pyrazinamide exposures in the CSF were strongly correlated to high mortality rates even when correcting for all other drug levels and CSF environment; This should be further investigated to establish whether a causative element exists in this strong correlation.

METHODS AND MATERIALS

Study design, setting and participants

The study was a randomised, double-blind, placebo-controlled trial with two parallel treatment arms – an immediate ART group (ART initiated within seven days of starting of TB treatment) and a deferred ART group (placebo treatment for two months followed by ART initiation after two months of TB therapy). The study was conducted at the Hospital for Tropical Diseases and at Pham Ngoc Thach Hospital, both in Ho Chi Minh City, Vietnam in 2011. The study participants were HIV-infected adults (aged > 15 years) who fulfilled diagnostic criteria for TBM. Baseline demographic clinical and laboratory features of the study participants are reported elsewhere²⁸. Rich pharmacokinetic data

were available for 44-46 patients for each of the four first-line anti-TB drugs. All samples were collected at steady state between days 8 and 55. Cerebrospinal fluid (CSF) data were available for 80-85 patients for each drug, including all of those who participated in the rich PK study and were collected at the same time as blood samples which is during steady state between days 8 and 55. Further information about TB diagnostic criteria, ethics review and informed consent can be found in the previously published full study²⁸.

Drug Dosing

Adults not previously treated for TB were given an initial dose of oral INH of 5mg/kg (maximum dose, 300mg/day), RIF 10mg/kg (maximum dose, 600mg/kg), PZA 25mg/kg (maximum dose, 2g/d), and ETB 20mg/kg (maximum dose, 1.2g/d), once daily for 3 months. After the initial three months PZA and ETB were stopped and RIF and INH maintenance therapy was continued for a further six months. Streptomycin, administered intramuscularly (20mg/kg; maximum dose, 1g/d) was added to the initial treatment regime of patients previously treated for TB. All patients received dexamethasone at an initial dose of 300-400 µg/kg/d unless it was contraindicated. ART or placebo tablets were given as soon as possible and no later than one week after randomisation. The ART regime constituted of oral zidovudine (GSK) at a twice daily dose of 300mg, lamivudine (GSK) at a twice daily dose of 150mg, and efavirenz (Merck) at a once daily oral dose of 800mg (if receiving rifampicin) or 600mg (if not receiving rifampicin). After two months all patients received ART until the end of the study (12 months).

Bioanalytical Methods

All plasma aliquots underwent protein precipitation with 200 µL AcN/MeOH 50/50 containing the appropriate internal standard as shown below. For RIF, ETB and INH, the HPLC system was interfaced with a triple-quadrupole TSQ Quantum Access mass spectrometer (Thermo Scientific, Hemel Hempstead, UK) with an electrospray ionization (ESI) source. For PZA, it was interfaced with a Shimadzu LC 2010 system with UV detection operated at 268 nm. All analytical methods were fully validated to international standards³⁷.

The mobile phases, HPLC columns and internal standards used for each compound were as follows:

RIF: Separation performed on a reverse-phase Hypersil-Hypurity C18 column (150 x 2.1 mm, 5 µm particle size - Thermo Scientific, UK) with a mobile phase of acetonitrile (ACN) containing formic acid (0.05% v/v) and 15 mM ammonium formate buffer (pH 5.0) under a gradient programme of 35-90%

ACN from 0-4 min, increasing to 95% ACN from 4 to 5 min, and finally reverting to 35% ACN from 5 to 6 min at a 350 $\mu\text{L}/\text{min}$ flow rate. Internal standard: rifapentine 250ng/ml.

INH & ETB: Chromatographic separation using a Hypersil GOLD C18 (150 x 4.6 mm, 3 μm particle size) column (Thermo Scientific, UK) operated at 30°C with an isocratic mobile phase of 90% water, 10% methanol and 0.3% formic acid at a 300 $\mu\text{L}/\text{min}$ flow rate. Internal Standard: metformin at 200 ng/mL.

PZA: Separation using Shimadzu LC 2010 HT HPLC system (Shimadzu, Manchester, UK) with an isocratic mobile phase of 95% water (0.06% TFA) and 5% ACN at a 1 mL/min flow rate. Internal Standard: acetazolamide at 10 $\mu\text{g}/\text{mL}$.

Further information about the bioanalytical methods are available in supplementary materials.

Pharmacokinetic analysis

PK analysis was performed using the Non-Parametric Adaptive Grid algorithm within the Pmetrics package (version 1.2.9)³⁸. A one compartment absorption model was sufficient to fit all data for all four drugs without the need of an extra equilibrating compartment. Exposure profiles were defined with three parameters (Absorption rate (K_a), Clearance rate (CL/F) and Volume of distribution (V/F)). The goodness of fit of the final pharmacokinetic model was determined by the precision of the parameter estimates and by visual examination of the scatter plot of residuals versus simulated predicted concentrations.

Covariates and Statistical Analysis

The covariates investigated were body weight, ART initiation time, CSF concentration at two hours after dose, age, sex, mortality within one year, and adverse neurological events. All covariate analyses were performed post-hoc using posterior estimates of the PK parameters as obtained from Pmetrics using SPSS 22.0 (IBM corp., Armonk, N.Y., U.S.A). Bivariate correlations with Spearman's correlation coefficients were used with a matrix containing all the mentioned covariates as well as the generated PK parameter estimates.

For the correlations that were significant in bivariate analyses between drug levels in the CSF and neurotoxicity/mortality, binomial logistic regression was used including all factors relating to the CSF environment including CSF lymphocyte count, CSF protein levels, CSF glucose levels, CSF neutrophil counts as well as CSF levels for all other drugs.

41-46 patients were available for rich pharmacokinetic data and 82-85 patients for CSF exposure data which allows for at least 80% power to discern a 36% difference in AUC between the placebo

and immediate ART groups in systemic PK exposure and a 25% difference in CSF exposure between the two groups in with a 2-sided significance level of 5%.

STUDY HIGHLIGHTS

- **What is the current knowledge on the topic?**

The clinical outcomes from immediate ART compared to delayed ART in patients diagnosed with TB and HIV have been studied and shown to have little effect upon mortality overall. The pharmacokinetics of TB drugs have been previously studied in other populations but not in TBM/HIV infected populations.

- **What question did this study address?**

Are the current dosing regimens in patients with concomitant TBM and HIV adequate?

- **What this study adds to our knowledge?**

The study gives strong indications that this TBM/HIV +ve population is prone to lower Rifampicin (RIF) exposure. It also shows a strong correlation between CSF exposure to Pyrazinamide (PZA) and mortality rates. Additionally, the study shows no significant effect of ART initiation time upon TB drug pharmacokinetics.

- **How this might change clinical pharmacology or translational science?**

The dosing of RIF in this population will have to be revised and changed to achieve at least exposures that are equivalent to other populations. Dosing of PZA should be questioned if the correlation with mortality turns out to be a causative one in this population.

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CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

G.G.A. and S.A.W. wrote the manuscript; E.T. and J.F. designed the research; G.G.A., E.T., T.T.H.C., N.T.H.M., N.H.P., and T.T.H. performed the research; G.G.A., D.W., and W.H. analyzed the data.

Accepted Article

LEGENDS FOR FIGURES

Figure 1.

Observed data in all patients for Ethambutol (ETB) (A), Isoniazid (INH) (B), Pyrazinamide (PZA) (C) and Rifampicin (RIF) (D) expressed as time after dose. The solid line represents the median of Monte-Carlo simulations performed on 1000 subjects given the estimated parameter values and their correlation matrix, while the dashed lines represent the 5% and 95% percentiles of these simulations.

Figure 2.

- A. Influence of ART initiation time upon drug exposure in the systemic circulation as defined by its CL/F (L/hr) value. ART initiation time does not influence exposure of ETB, INH, PZA or RIF (Spearman's correlation, $P > 0.05$, $n = 19-27$).
- B. Influence of ART initiation time upon CSF bioavailability as defined by CSF drug concentration (ng/mL) at 2 hrs post dose. ART initiation time does not influence exposure of ETB, PZA or RIF but delayed ART significantly correlates with increased INH exposure in the CSF (Spearman's correlation, $P < 0.05$, $n = 20-46$).

Figure 3.

- A. Correlation between PZA CSF exposure (as defined by CSF drug concentration @ 2 hours post dose) and mortality (Defined as death or survival in 1 year). Higher CSF PZA exposure significantly correlates with mortality rates (Spearman's correlation, $P < 0.005$, $n = 30-34$)
- B. Correlation between PZA CSF exposure (as defined by CSF drug concentration @ 2 hours post dose) and neurological events (defined in text). Higher CSF PZA exposure significantly correlates with neurological events (Spearman's correlation, $P < 0.05$, $n = 24-40$)

Figure 4.

Monte-Carlo simulations of RIF exposure in HIV –ve subjects (based on literature values) compared to A. current cohort with the actual 5mg/kg dose or B. current cohort with a suggested 900mg RIF tablet.

1. Rieder HL, Snider DE, Jr., Cauthen GM. Extrapulmonary tuberculosis in the United States. *The American review of respiratory disease* 1990; **141**(2): 347-51.
2. WHO. Global tuberculosis report 2016. Geneva, Switzerland, 2016.
3. Thwaites GE, Nguyen DB, Nguyen HD, et al. Dexamethasone for the treatment of tuberculous meningitis in adolescents and adults. *The New England journal of medicine* 2004; **351**(17): 1741-51.
4. Rock RB, Olin M, Baker CA, Molitor TW, Peterson PK. Central nervous system tuberculosis: pathogenesis and clinical aspects. *Clinical microbiology reviews* 2008; **21**(2): 243-61, table of contents.
5. Corbett EL, Watt CJ, Walker N, et al. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Archives of internal medicine* 2003; **163**(9): 1009-21.
6. Thwaites GE, Duc Bang N, Huy Dung N, et al. The influence of HIV infection on clinical presentation, response to treatment, and outcome in adults with Tuberculous meningitis. *The Journal of infectious diseases* 2005; **192**(12): 2134-41.
7. Lawn SD, Myer L, Bekker LG, Wood R. Tuberculosis-associated immune reconstitution disease: incidence, risk factors and impact in an antiretroviral treatment service in South Africa. *Aids* 2007; **21**(3): 335-41.
8. Abdool Karim SS, Naidoo K, Grobler A, et al. Timing of initiation of antiretroviral drugs during tuberculosis therapy. *The New England journal of medicine* 2010; **362**(8): 697-706.
9. Bhatt NB, Barau C, Amin A, et al. Pharmacokinetics of rifampin and isoniazid in tuberculosis-HIV-coinfected patients receiving nevirapine- or efavirenz-based antiretroviral treatment. *Antimicrobial agents and chemotherapy* 2014; **58**(6): 3182-90.
10. Chideya S, Winston CA, Peloquin CA, et al. Isoniazid, rifampin, ethambutol, and pyrazinamide pharmacokinetics and treatment outcomes among a predominantly HIV-infected cohort of adults with tuberculosis from Botswana. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2009; **48**(12): 1685-94.
11. Perlman DC, Segal Y, Rosenkranz S, et al. The clinical pharmacokinetics of rifampin and ethambutol in HIV-infected persons with tuberculosis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2005; **41**(11): 1638-47.
12. Asproдини EK, Zifa E, Papageorgiou I, Benakis A. Determination of N-acetylation phenotyping in a Greek population using caffeine as a metabolic probe. *European journal of drug metabolism and pharmacokinetics* 1998; **23**(4): 501-6.
13. D'Oliveira JJ. Cerebrospinal fluid concentrations of rifampin in meningeal tuberculosis. *The American review of respiratory disease* 1972; **106**(3): 432-7.
14. Jonsson S, Davidse A, Wilkins J, et al. Population pharmacokinetics of ethambutol in South African tuberculosis patients. *Antimicrobial agents and chemotherapy* 2011; **55**(9): 4230-7.
15. Israili ZH, Rogers CM, el-Attar H. Pharmacokinetics of antituberculosis drugs in patients. *Journal of clinical pharmacology* 1987; **27**(1): 78-83.
16. Lee CS, Gambertoglio JG, Brater DC, Benet LZ. Kinetics of oral ethambutol in the normal subject. *Clinical pharmacology and therapeutics* 1977; **22**(5 Pt 1): 615-21.
17. McIlleron H, Wash P, Burger A, Norman J, Folb PI, Smith P. Determinants of rifampin, isoniazid, pyrazinamide, and ethambutol pharmacokinetics in a cohort of tuberculosis patients. *Antimicrobial agents and chemotherapy* 2006; **50**(4): 1170-7.
18. Babalik A, Ulus IH, Bakirci N, et al. Pharmacokinetics and serum concentrations of antimycobacterial drugs in adult Turkish patients. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2013; **17**(11): 1442-7.
19. Tostmann A, Mtabho CM, Semvua HH, et al. Pharmacokinetics of first-line tuberculosis drugs in Tanzanian patients. *Antimicrobial agents and chemotherapy* 2013; **57**(7): 3208-13.

20. Kergueris MF, Bourin M, Larousse C. Pharmacokinetics of isoniazid: influence of age. *European journal of clinical pharmacology* 1986; **30**(3): 335-40.
21. Burhan E, Ruesen C, Ruslami R, et al. Isoniazid, rifampin, and pyrazinamide plasma concentrations in relation to treatment response in Indonesian pulmonary tuberculosis patients. *Antimicrobial agents and chemotherapy* 2013; **57**(8): 3614-9.
22. De-jian S, Xu-li D, Ji-hui D. The history of the elimination of lymphatic filariasis in China. *Infectious diseases of poverty* 2013; **2**(1): 1.
23. Lacroix C, Hoang TP, Nouveau J, et al. Pharmacokinetics of pyrazinamide and its metabolites in healthy subjects. *European journal of clinical pharmacology* 1989; **36**(4): 395-400.
24. Loos U, Musch E, Jensen JC, Mikus G, Schwabe HK, Eichelbaum M. Pharmacokinetics of oral and intravenous rifampicin during chronic administration. *Klinische Wochenschrift* 1985; **63**(23): 1205-11.
25. Milan Segovia RC, Dominguez Ramirez AM, Jung Cook H, et al. Population pharmacokinetics of rifampicin in Mexican patients with tuberculosis. *Journal of clinical pharmacy and therapeutics* 2013; **38**(1): 56-61.
26. Ruslami R, Nijland HM, Alisjahbana B, Parwati I, van Crevel R, Aarnoutse RE. Pharmacokinetics and tolerability of a higher rifampin dose versus the standard dose in pulmonary tuberculosis patients. *Antimicrobial agents and chemotherapy* 2007; **51**(7): 2546-51.
27. Blanc FX, Sok T, Laureillard D, et al. Earlier versus later start of antiretroviral therapy in HIV-infected adults with tuberculosis. *The New England journal of medicine* 2011; **365**(16): 1471-81.
28. Torok ME, Yen NT, Chau TT, et al. Timing of initiation of antiretroviral therapy in human immunodeficiency virus (HIV)--associated tuberculous meningitis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2011; **52**(11): 1374-83.
29. Reynolds J, Heysell SK. Understanding pharmacokinetics to improve tuberculosis treatment outcome. *Expert opinion on drug metabolism & toxicology* 2014; **10**(6): 813-23.
30. Rastogi N, Labrousse V, Goh KS. In vitro activities of fourteen antimicrobial agents against drug susceptible and resistant clinical isolates of Mycobacterium tuberculosis and comparative intracellular activities against the virulent H37Rv strain in human macrophages. *Current microbiology* 1996; **33**(3): 167-75.
31. Donald PR. Cerebrospinal fluid concentrations of antituberculosis agents in adults and children. *Tuberculosis* 2010; **90**(5): 279-92.
32. Pouplin T, Bang ND, Toi PV, et al. Naïve-pooled pharmacokinetic analysis of pyrazinamide, isoniazid and rifampicin in plasma and cerebrospinal fluid of Vietnamese children with tuberculous meningitis. *BMC infectious diseases* 2016; **16**(1): 144.
33. Saleri N, Dembélé SM, Villani P, et al. Systemic exposure to rifampicin in patients with tuberculosis and advanced HIV disease during highly active antiretroviral therapy in Burkina Faso. *Journal of Antimicrobial Chemotherapy* 2012; **67**(2): 469-72.
34. Patel KB, Belmonte R, Crowe HM. Drug malabsorption and resistant tuberculosis in HIV-infected patients. *The New England journal of medicine* 1995; **332**(5): 336-7.
35. Heemskerk AD, Bang ND, Mai NT, et al. Intensified Antituberculosis Therapy in Adults with Tuberculous Meningitis. *New England Journal of Medicine* 2016; **374**(2): 124-34.
36. Schaberg T, Rebhan K, Lode H. Risk factors for side-effects of isoniazid, rifampin and pyrazinamide in patients hospitalized for pulmonary tuberculosis. *The European respiratory journal* 1996; **9**(10): 2026-30.
37. Guidances (Drugs). In: Administration) USFaD, editor.; 2015.
38. Neely MN, van Guilder MG, Yamada WM, Schumitzky A, Jelliffe RW. Accurate detection of outliers and subpopulations with Pmetrics, a nonparametric and parametric pharmacometric modeling and simulation package for R. *Therapeutic drug monitoring* 2012; **34**(4): 467-76.

Supplementary Materials

Table 1 Total number of subjects enrolled in the PK study, total number of concentrations, median and range of concentration numbers available per person and % patients who had immediate concomitant ART.

LC-MS/MS method for the determination of rifampicin

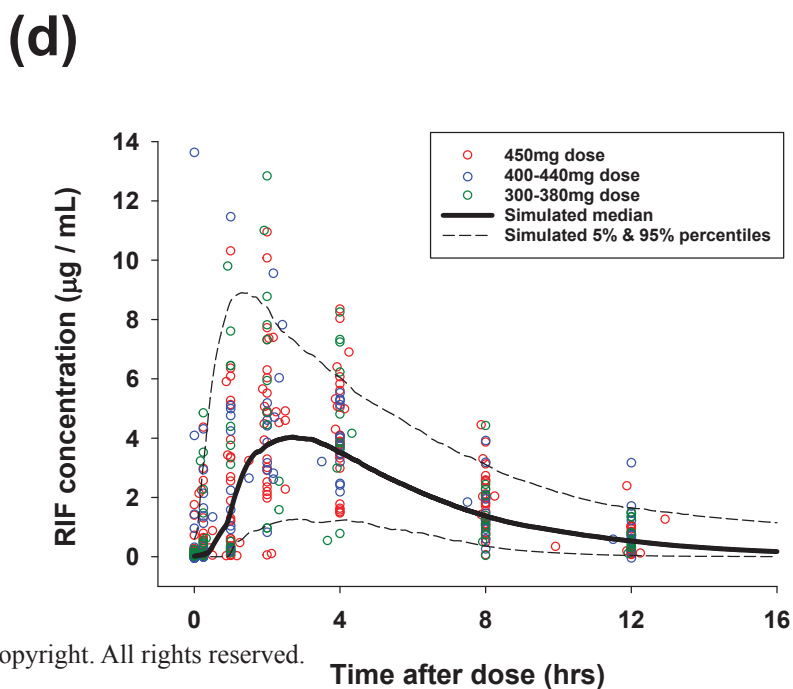
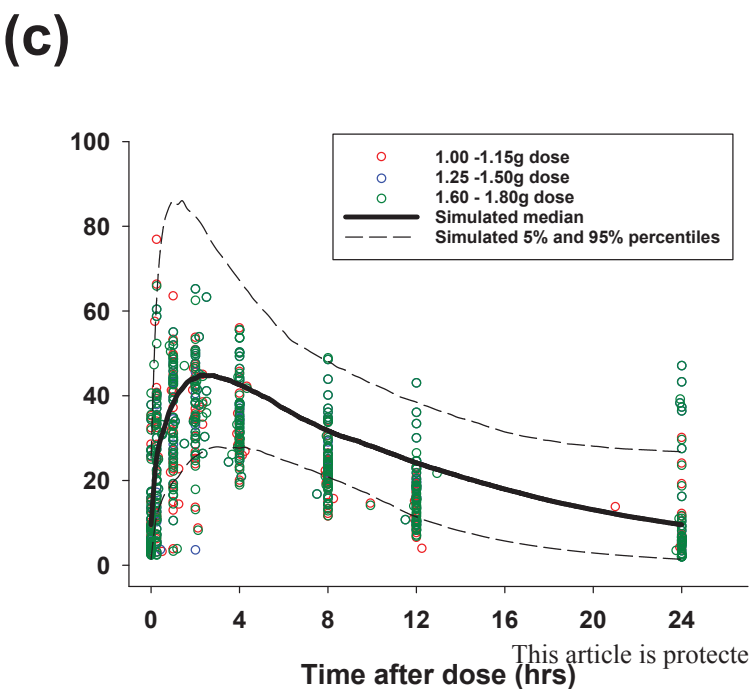
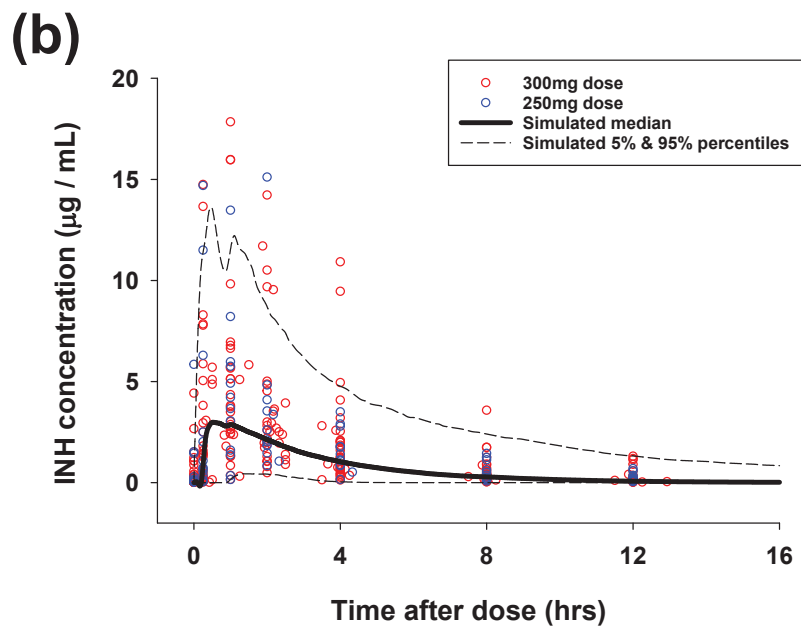
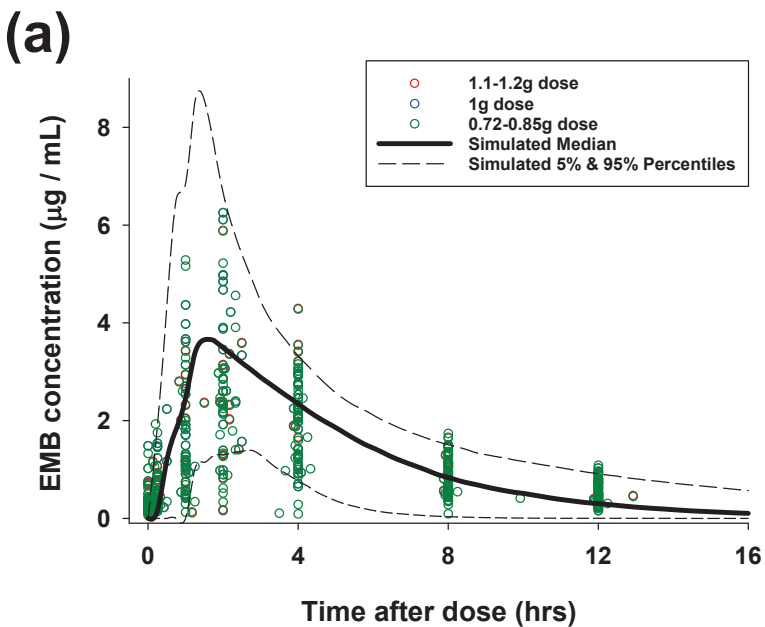
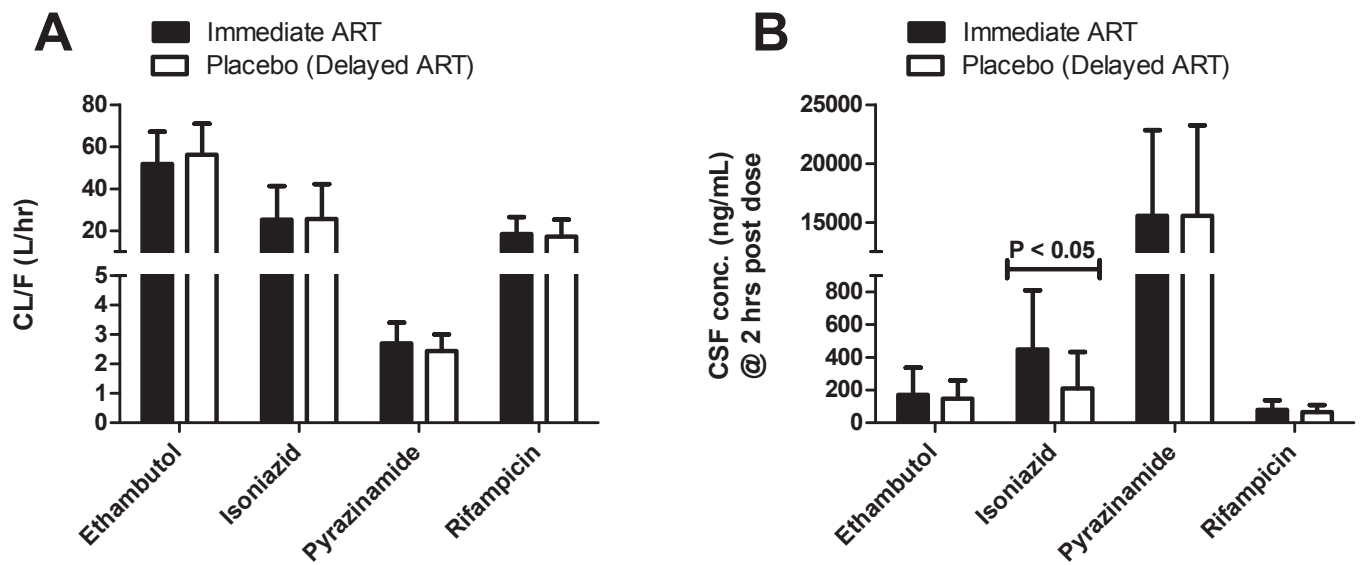


Table 1 Population mean values and variance of major PK parameters (CL/F, V/F, k_a , and Lag time) for ETB, INH, PZA and RIF. Values were generated using a one compartment model with an absorption lag time using Pmetrics®.

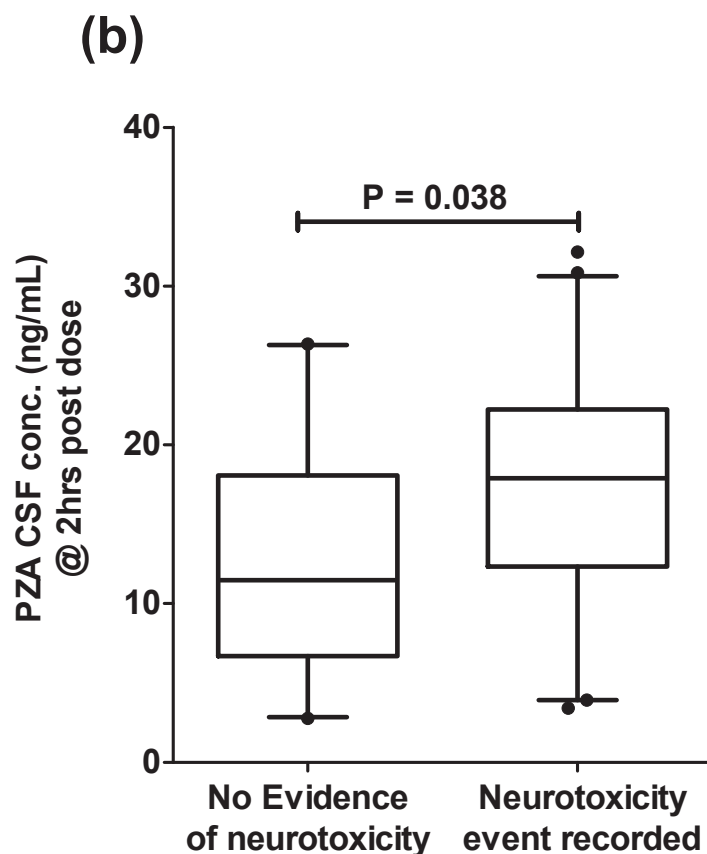
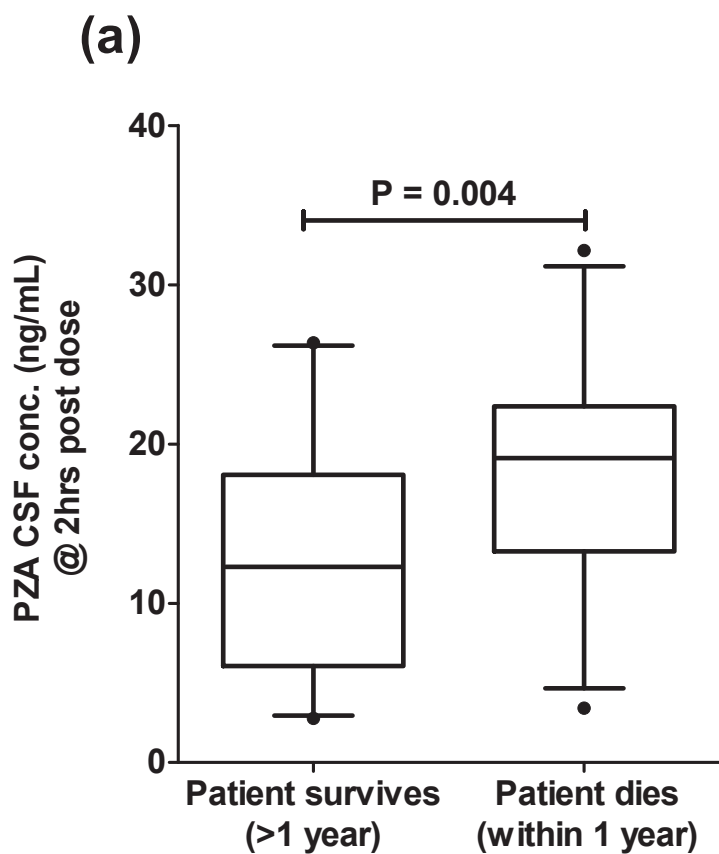
ETB		
Parameter	Pop. Mean	S.D. (CV%)
CL/F (L/hr)	53.6	15.8 (29.83%)
V/F (L)	135.3	82.0 (60.62%)
k_a (hr ⁻¹)	2.3	2.9 (127.9%)
Absorption Lag (hrs)	0.35	0.36 (104.5%)
AUC (24 hrs @ SS) (µg.hr/mL)	19.2	5.6 (29.3%)
CSF conc. 2hr post dose (ng/mL)	160.8	146.0 (90.7%)
INH		
Parameter	Pop. Mean	S.D. (CV%)
CL/F (L/hr)	25.4	16.9 (66.6%)
V / F (L)	93.9	127.5 (135.0%)
k_a (hr ⁻¹)	8.1	6.1 (75.6%)
Absorption Lag (hrs)	0.20	0.28 (135.2%)
AUC (24 hrs @ SS) (µg.hr/mL)	17.5	14.3 (81.5%)
CSF conc. 2hr post dose (ng/mL)	347.1	329.1 (94.8%)
PZA		
Parameter	Pop. Mean	S.D. (CV%)
CL/F (L/hr)	2.6	0.70 (27.10%)
V/F (L)	48.5	54.2 (111.8%)
k_a (hr ⁻¹)	2.7	2.0 (74.80%)
Absorption Lag (hrs)	N/A	N/A
AUC (24 hrs @ SS) (µg.hr/mL)	489.5	126.0 (25.7%)
CSF conc. 2hr post dose (µg/mL)	15.6	7.4 (47.4%)
RIF		
Parameter	Pop. Mean	S.D. (CV%)
CL/F (L/hr)	17.9	8.2 (46.2%)
V/F (L)	53.6	35.1 (65.5%)
k_a (hr ⁻¹)	0.92	0.85 (92.7%)
Absorption Lag (hrs)	0.52	0.42 (80.9%)
AUC (24 hrs @ SS) (µg.hr/mL)	29.8	13.8 (46.5%)
CSF conc. 2hr post dose (ng/mL)	73.1	51.3 (70.2%)



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Table 2 Literature estimates of clearance values of ETB, INH, PZA and RIF compared to estimates in current study. Most estimates in the literature are of HIV –ve/TB +ve patients.

CL/F (L/hr)	Population	Reference
ETB		
53.6	TBM/HIV +ve adults (Vietnamese)	Current study
40.3	TB +ve adults (South African)	14
67	TB +ve adults	15
43	Healthy adults	16
70	TB +ve adults	17
INH		
25.4	TBM/HIV +ve adults (Vietnamese)	Current study
15	TB +ve adults	15
18	TB +ve adults (Turkish)	18
25.8	TB +ve adults (Tanzanian)	19
11.2	TB +ve adults	20
PZA		
2.6	TBM/HIV +ve patients (Vietnamese)	Current study
2.61	TB +ve adults (Indonesian)	21
4.5	TB +ve adults (Tanzanian)	19
1.9	Healthy adults (Chinese)	22
3.6	Healthy adults	23
RIF		
17.9	TBM/HIV +ve adults (Vietnamese)	Current study
14.20	TB +ve adults	24
10.2	TB +ve adults (Turkish)	18
8.17	TB +ve adults (Mexican)	25
7.5-9.2	TB +ve adults (Indonesian)	26



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Table 3 Spearman's correlation between Drug exposure as defined by AUC (24 hours at steady state) or CSF bioavailability (CSF conc. @ 2 hrs post dose) with Time of ART initiation, patient weight, sex, age, HIV severity (defined by CD4 counts and HIVRNA counts)

Drug	Parameter	Statistic	AUC _{ss}	ART initiation time	Sex	Age	Weight	CSF conc. @ 2hrs	CD4 count	HIVRNA	Death within 9 months	Death within 3 years	Total time till Death	Time till Neurological Event
ETB	AUC _{ss}	Cor. Coef.	1.000	-.199	-.001	-.069	.038	.217	-.016	-.156	.003	.010	-.039	-.101
		P value		.185	.995	.649	.803	.161	.918	.302	.985	.945	.799	.505
		N	46	46	46	46	46	43	46	46	46	46	46	46
RIF	AUC _{ss}	Cor. Coef.	1.000	-.016	-.034	.054	.031	-.168	.121	.062	-.255	-.318	.268	-.108
		P value		.921	.829	.725	.841	.321	.434	.690	.103	.061	.086	.251
		N	44	44	44	44	44	40	44	44	44	44	44	44
INH	AUC _{ss}	Cor. Coef.	1.000	-.038	-.085	-.065	-.287	.735**	.143	.002	.026	.040	-.274	-.024
		P value		.811	.592	.684	.065	.000	.368	.991	.870	.799	.080	.878
		N	42	42	42	42	42	21	42	42	42	42	42	42
PZA	AUC _{ss}	Cor. Coef.	1.000	.080		.169	.151	.105	-.109	.318	.187	.139	-.078	-.056
		P value		.603		.268	.323	.526	.475	.033	.219	.361	.609	.714
		N	45	45	45	45	45	39	45	45	45	45	45	45
ETB	CSF conc.	Cor. Coef.	.217	-.096	.072	.097	-.022	1.000	.142	.093	.114	.116	-.266*	-.358**
		P value	.161	.395	.528	.391	.847		.210	.413	.315	.304	.017	.001
		N	43	80	80	80	80	80	80	80	80	80	80	80
RIF	CSF conc.	Cor. Coef.	-.168	-.112	-.006	.032	-.162	1.000	.242	-.021	-.129	-.141	.062	-.023
		P value	.321	.336	.960	.786	.161		.035	.858	.268	.223	.596	.844
		N	40	76	76	76	76	76	76	76	76	76	76	76
INH	CSF conc.	Cor. Coef.	.735**	-.352*	.079	-.174	-.196	1.000	-.004	.098	.063	.063	-.162	-.133
		P value	.000	.015	.599	.241	.187		.978	.512	.675	.675	.277	.373
		N	21	47	47	47	47	47	47	47	47	47	47	47
PZA	CSF conc.	Cor. Coef.	.105	.010	.247*	-.122	-.153	1.000	-.021	.000	.373**	.354**	-.470**	-.372**
		P value	.526	.936	.049	.335	.227		.868	1.000	.002	.004	.000	.002
		N	39	64	64	64	64	64	64	64	64	64	64	64

